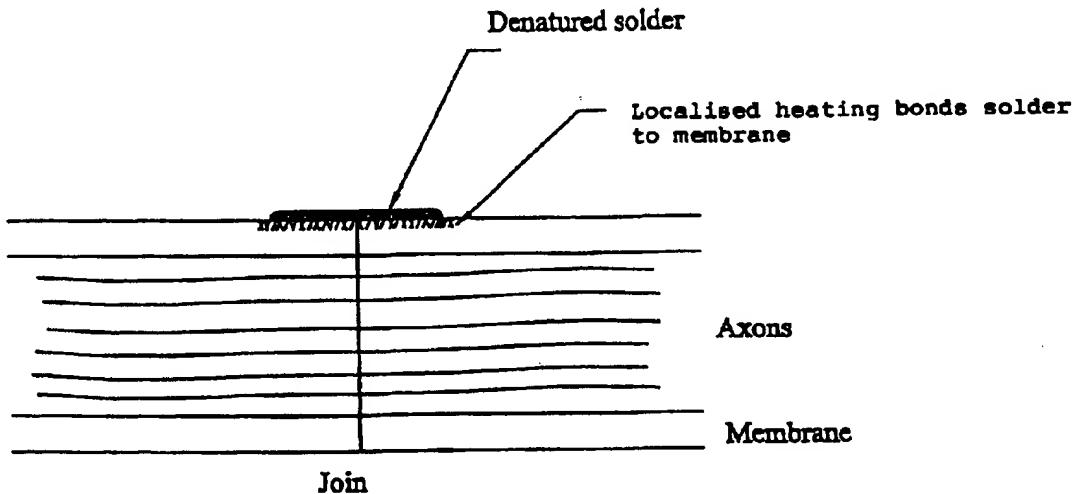




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(54) Title: METHOD OF TISSUE REPAIR



(57) Abstract

A method for joining tissue comprising aligning and abutting edges of the tissue to be joined applying a biodegradable, biological solder or an analogue thereof, across the edges and exposing the solder to an energy source under conditions which provide transfer of energy from the source to the solder to cause the solder to bond to the tissue surface adjacent the edges to provide a weld holding the edges together.

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METHOD OF TISSUE REPAIR

TECHNICAL FIELD

The present invention relates to methods for joining living tissues, including veins, arteries, microvessels, 5 tubes, nerves, organ tissues and biological surfaces, such as peritoneum, omentum, fascia, skin, artificial tissues, and to pharmaceutical products useful in joining these tissues.

BACKGROUND ART

10 Joining tissues such as veins, arteries, microvessels, tubes, nerves, tissues and biological surfaces such as the peritoneum and skin has mainly been carried out clinically to date by suturing and microsuturing.

15 Microsuturing requires considerable skill and is a time consuming procedure. Frequently, tissues which have been joined by microsuturing form considerable scar tissue. Some of the difficulties encountered with microsuturing can be better understood by considering the 20 example of rejoining damaged peripheral nerve tissue.

Peripheral Nerves

The electrical signals that control the body's organs and transmit information back and forth to the central nervous system (CNS) travel along peripheral 25 nerves. The structure of these peripheral nerves is analogous to telephone cables. In a telephone cable there is a strong protective outer coating that protects all the inner components. The copper wires are often grouped in separate insulating tubes that lead to 30 different systems. Each of the inner copper wires is a single line that can transmit electricity in either direction and has an insulating coating around it so that it does not interfere with the lines next to it.

A peripheral nerve (Figure 1) has an outer membrane 35 consisting of connective tissue such as collagen. This

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membrane (epineurium) protects and holds the separate nerve bundles together. The nerve bundles which lie inside this membrane are called fascicles. These fascicles also have a collagen based surrounding membrane and their task is to group together nerve axons supplying a similar area of the body. Inside the fascicle membrane the axons are surrounded by loose connective tissue. The axons are a long extension from a cell body which is contained within the CNS in the spine or the brain.

10 Sensory axons transmit to the CNS and motor axons transmit from the CNS. Nerve metabolism is sustained by the vascular system from both outside the nerve and along the centre of the nerve.

Peripheral nerves can have very small diameters. For instance, the mature median nerve at the wrist is approximately 1 cm in diameter and contains an average of forty fascicles, each of which can contain up to 4500 axons. When a peripheral nerve is cut all axons distal to the wound change their properties as axon flow is cut off from the cell body. Even when the nerve is reconnected, these axons continue to degenerate distally. The Schwann cells which normally wrap themselves around the axons as insulation guide regenerating axons. Joining nerves as accurately as possible by lining up corresponding fascicles enables the axons to more efficiently regenerate.

Operating upon nerves has been facilitated by using magnification and special microsurgical equipment. Accurate repairs need to be effected at the fascicular level ensuring that regeneration is along the correct bundle leading to the original area those axons supplied. The current technique of peripheral nerve repair uses microsuturing (Figure 2). This technique requires a dedicated, trained surgeon as microsuturing of just one of the many fascicles with three or more microsutures (using say a 70 micron diameter needle and 30 micron thread) can take very long operating times.

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Microsuturing is at present clinically used where the skills are available. Unfortunately, there are relatively few surgeons who have the necessary manipulative skills for operating at high magnification.

5 Even a reasonable microsuturing technique results in long operating times with added damage to the inner axons due to sutures penetrating the thin insulating perineurial sheath. The use of sutures results in some scarring of the repair due to foreign body reaction. There is also 10 evidence which indicates that in the long term scar tissue formation and scar maturation can lead to impairment of the joined nerve.

Work has been performed on the use of lasers alone in effecting nerve joins. One of the problems of laser welding has been the fact that the intact gel-like nerve tissue of the axons is actually under pressure within the fascicle. When the fascicle is cut this material extrudes. This can lead to the direct laser weld being formed on nerve tissue rather than the surrounding 20 membrane of the fascicle, causing nerve damage. To date the welds have typically been made using infrared lasers such as CO₂ lasers which rely on water absorption for energy transfer. Tissue preparation before welding relies on overlapping the nerve membranes. This is 25 difficult due to the extruding gel-like axons and so can lead to denaturation of the nerve axon material. The affected tissue tends to scar and the fibrous tissue that proliferates as a result is a poorer electrical conductor than nerve tissue. The bonds formed to date as described 30 in the prior art using laser welding have typically lacked strength. These laser joins alone tend to fail so microsuturing has been used in addition to welding to strengthen these joins.

To deal with at least some of the deficiencies of 35 laser welding, various glues have been used in forming the welds. These low protein concentration, fluid glues tend to run between the ends of the nerve that are being joined which may result in damage to the axoplasm of the

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nerve fascicle and also hinder regeneration. They are also applied around the join which is then circumferentially welded. These joins later show thick scarring which causes stricture of the nerve. Moreover, 5 the joins tend to be weak.

The welding techniques so far available also tend to lack precision. Factors that influence the precision of this approach adversely include differences in: the consistency of the glue used; the aperture of the needle 10 or other device used to apply the glue; and the pressure exerted in applying the glue.

DESCRIPTION OF THE INVENTION

The present invention provides a method for joining tissue comprising:

15 aligning and abutting edges of the tissue to be joined;

applying a solder, across the aligned and abutted edges; and

20 exposing the solder to an energy source under conditions which provide a transfer of energy from the source to the solder to cause the solder to bond to the tissue surface adjacent the edges thus providing a weld holding the edges together.

In addition to causing the solder to bond to the 25 protein of the underlying tissue, the energy transfer can affect the structure of the solder itself leading to bonding within the solder and an enhancement of the strength of the solder and hence the join.

Drops of solder are typically used where the solder 30 is a fluid solder, and are "painted" across the edges.

The solder can also be provided as a preformed solid strip.

The energy source is typically a laser.

A variety of tissue types can be joined using this 35 method. The method is applicable to anastomoses of biological tubes including veins, arteries, lymphatics, nerves, vasa efferentia, fallopian tubes, bile ducts,

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tubes of the alimentary canal, the ureter, the urethra, tear ducts, bronchi and any other such bodily tubes as well as to repairs of incisions or tears of biological organs such as kidneys, liver or spleen, or of biological 5 surfaces such as the peritoneum and skin. It will therefore be understood that the method can be used in a variety of join situations including the joining of cylindrical anastomoses and the closure of linear defects such as incisions.

10 Where the tissue repair is with respect to nerve tissue or other tissue tubes where the tube contents need to be protected from damage, it is especially important that the weld should not be concentrated on the edges being joined as this can damage extruded tissue. Rather, 15 the weld should be distributed across the planar or tubular surface in which the discontinuity lies.

Where the tissue to be repaired is an essentially hollow body tube such as a blood vessel, the repair can additionally comprise the insertion of a thin-walled 20 hollow cylinder of solder inside the tube under repair so that the cylinder spans the severed portions of the tube. Typically, while the severed tube and cylinder assembly 25 is held together, energy from the energy source is directed through the tube wall to bond the cylinder to the tube ends. The cylinder may incorporate a dye, as hereinafter described, to attract energy to the cylinder for more efficient welding. The repair is completed by the application of at least one strip or drop of solder 30 across the edges on the outer surface and treating the applied solder as described above.

Where the repair is with respect to tissue surfaces such as peritoneum, it will be understood that it is less important to avoid concentration of welding on the edges.

The method can also be modified for the repair of 35 other discontinuities in tissue surfaces such as holes, resulting from accident or surgery. In this form of the invention the solder may be spread or pre-cut to conform to the shape of the repair site, and the edges of the

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repair site may not need to be aligned or abutted for the repair to be effected.

A typical nerve repair using the method of the invention is one in which the edges are ends of a cut 5 peripheral nerve fascicle that are to be joined together or an end of a nerve fascicle and the fascicle of substitute nerve graft material. This latter situation is particularly applicable where nerve repair is required but a section of the nerve under repair has been severely 10 damaged or is unavailable, so that the available ends of the fascicle are too remote from each other to be directly joined. The actual nature of the damage sustained by the nerve and whether the repair is a primary or secondary repair are factors affecting 15 recovery but in any case the edges of nerve fascicles to be joined are cleanly cut at right angles prior to joining.

Application of the solder as a strip or strips, with space between for natural co-apportion of the surfaces 20 themselves permits the nerve under repair to revascularise. Circumferential welding, by comparison, can inhibit the body's natural healing process and so slow down blood capillary access needed for the area of repair. Laser soldering and suturing techniques 25 ultimately rely on the body regenerating connective tissue to hold the nerve together after either solder or suture connections break down and are replaced by the healing process. The present inventors have shown in *in vivo* experiments that successful regeneration can be 30 achieved by the methods of the present invention without restriction on surrounding tissue movement after the operation. In the case of nerve repair operation on human patients it is routine to initially restrict the movements of the joints of the operated limbs to assist 35 in reducing tension across the repair site.

Typical biodegradable, biological solders useful in the method of the invention include protein solders.

It is envisaged that other naturally occurring biomolecules could be used as alternatives. Further analogues of biological, biodegradable polypeptides could be used. Analogues of biological, biodegradable 5 polypeptides useful in the invention include synthetic polypeptides and other molecules capable of forming a viscous "glue" that does not react adversely within the tissue undergoing repair.

10 The protein solder may be a solid or a fluid solder composition.

Fluid protein solder compositions useful in strip welding typically comprise between 100 and 120 mass % of protein relative to water. Preferably, fluid protein solders comprise between 100 and 110 mass % protein 15 relative to water.

The fluid solder strip is typically 50 to 200 μ m in thickness. Its length is selected to suit the join to be formed but typically is of the order of 2 to 3 mm in length. It is typically painted across the join.

20 Solid protein solder compositions useful in strip welding typically comprise between 120 and 230 mass % protein relative to water. Preferably the strip comprises 170 to 230 mass % protein and more preferably about 210 mass %.

25 It will be understood that different proteins will have different degrees of solubility in water or appropriate solutions which in turn will affect the optimum concentration of protein in the composition for different protein solders. Appropriate ranges for 30 particular proteins in both solid and fluid solders can be determined based on the known properties of the proteins.

Typically, the solid protein solder composition is provided as a preformed strip. Solid solder strips are 35 easier to manipulate than fluid solders. Under the moist conditions inherent in surgery fluid solders may run making it difficult to laser denature the solder before it has spread. The solid solder strips can have a paste

like or more rigid consistency. They are typically placed across the join with microforceps. In one form of the invention, it is envisaged that the solder strips will be substantially rectangular in shape. However, 5 different shape strips may be required in different repair situations. It may also be desirable to provide a plurality of strips joined together for efficient repair of a large or a substantial number of repair sites.

10 The protein solder may comprise a single protein of which albumin is a typical example or alternatively the solder may comprise more than one protein.

15 Albumin has desirable qualities for solid solder strip formation since it has a high proportion of β sheet structure which gives rigidity to the strips. Fibrin is another example of a protein with significant β sheet structure. Incorporation of α helical protein in the solder can assist in making the strips more malleable and thus retain a flatter profile which is particularly well suited for joining nerve ends. An example of a suitable 20 proportion of α helical protein is between 1 and 10% by weight of the protein used. About 5% is a preferred amount. Collagen, tropoelastin and elastin are examples of suitable α helical proteins.

25 Protein used in the solder is selected to minimise the risk of adverse host reactions and should therefore preferably be an autologous protein for the host or a foreign protein of low antigenicity.

30 The proteins may be obtained from any suitable source. Recombinantly or synthetically produced proteins as well as purified naturally occurring proteins may be used.

35 Preferably, when the solder is to be used with a laser which produces energy at a suitable wavelength the composition includes a substance, such as a dye, which absorbs energy at the wavelength produced by the laser with which the solder is to be used. It is preferable to choose the combination such that the dye or other substance absorbs the energy transmitted by the laser

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efficiently but the underlying tissue to be joined absorbs the transmitted energy poorly. The dye or other substance assists in making the welding specific to the solder used which in turn assists in minimising 5 accidental tissue heating damage to the underlying tissue.

The process of bonding, where protein solders are used, relies on protein molecules being available for cross-linking. This occurs when the protein molecules 10 are unfolded. Upon laser irradiation of, for instance, an albumin and indocyanine green containing solder at a nerve tissue join, albumin molecules are heated through energy transfer from the indocyanine green molecules, allowing them to unfold and bond between themselves and 15 to neighbouring tissue surface such as the fascicle membrane.

Dyes which contrast with the tissues being repaired can also be useful in making the solder easier to see. An example of a dye with this property is indocyanine 20 green.

When the laser used is a CO₂ laser, a dye will not assist the energy transfer, as the energy transfer is by water absorption.

The energy provided by the energy source should be 25 sufficient to bond the solder to form the weld while minimising damage to the underlying tissue. The temperature required to denature a protein solder is typically at least 50°C and may exceed 100°C. A preferred range is 50° to 90°C. A particularly preferred 30 range is 80° to 90°C.

The time of treatment for each join to be effected can vary depending on such factors as ambient conditions, altitude, and of course the nature of the tissue to be joined. The duration of treatment is typically short. A 35 30 second passage for laser treatment of a 0.4 mg strip is an example of the time involved although it will be understood that shorter or longer treatment times could

be required. It will be understood that solid solder takes longer to denature than fluid solder.

In a second aspect the present invention provides a protein solder composition comprising protein and a 5 suitable solvent for the protein. Water is typically used as the solvent for water soluble proteins.

In a third aspect the present invention provides a kit for use in joining tissues comprising, in a 10 preferably sterile pack, a plurality of protein solder strips and/or shapes of the second aspect of the invention. Preferably a plurality of strip lengths and/or shape sizes are included in the pack.

The kit preferably includes means for sterile manipulation of the strips. The kit also preferably 15 includes means for measuring the strips.

The kit may also comprise an energy source such as a fibre coupled laser system.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the structure of a peripheral nerve 20 in schematic form.

Figure 2 shows the joining of a peripheral nerve by prior art microsuturing techniques.

Figures 3 a) and b) shows in schematic form joining of a nerve fascicle with a) fluid solder and b) solid 25 strips.

Figure 4 shows the repair site of a 0.3 mm diameter tibial nerve immediately after: a) diode laser strip welding, and b) microsuturing.

Figure 5a shows a rat tibial nerve welded by the 30 laser solder methods of the present invention. The solder and the membrane are denatured but no significant change to the axons has occurred (x100 Giemsa).

Figure 5b shows a rat sciatic nerve joined by 35 microsuturing using 10-0 nylon. Localised perineural and axonal damage occurs.

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Figure 6 shows in schematic form joining of a blood vessel using internal biodegradable solid solder cylinder and external solid solder strips.

Figure 7 shows in schematic form a cross-section of 5 a repaired nerve fascicle.

Figure 8 shows the method used for measuring tensile strength of repaired nerves.

Figure 9 shows a solid solder strip positioned upon a severed rat tibial nerve just prior to laser welding.

10 Figure 10a shows regeneration of myelinated axons in a laser nerve repair that has regenerated for 3 months.

Figure 10b shows fibrous tissue around a suture in a sutured nerve that has regenerated for 3 months.

15 Figure 11 shows muscle action potential results for repaired nerves.

BEST METHOD OF CARRYING OUT THE INVENTION

Tissue repair is performed using a laser to activate a protein solder applied across the tissue edges to be joined. This solder denatures upon laser irradiation and 20 bonds with itself and the neighbouring membrane to form the join. The procedure is shown schematically in Figures 3 and 7 for a repair to a nerve fascicle. The solder is applied in longitudinal strips across the join.

Nerve Repair

25 Repair to severed nerve tissues is effected by the placement of solder across the severed edges and exposure of the solder to laser as described above. In order to repair nerve tissue without damage to the contents of the nerve it is desirable to avoid concentrating the weld on 30 the edges as extruded nerve contents may be damaged. Rather the weld should be distributed across the planar or tubular surface in which the discontinuity lies.

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Hollow Body Tube Repair

When repairing hollow body tubes it is preferable to insert an internal cylinder of solder into the tube so that it lies between the discontinuity. The severed ends 5 of the tube are placed over opposite ends of the solder cylinder. The arrangement is shown in Figure 6. Lasering can then be effected to cause bonding of the cylinder to the tube being joined while the arrangement is held in place. If there is a good fit between the 10 tube and the cylinder this laser step may not be required. The join is completed by the addition of external solder as for nerve repairs.

Tissue Surface Repairs

Surfaces such as peritoneum are planes of tissue in 15 which joins without sutures can be achieved by the application of solder across the discontinuities to be joined and welding as described above. In this case it is less important to avoid concentration of welding on the edges.

20 Laser and Solder System Suited to Nerve Fasicular Repair

To denature the protein solder, a GaAs/GaAlAs laser diode with a nominal power of 250 mW (Spectra Diode Labs, San Jose, California) is used. The laser light is coupled into a 100 μm diameter core optical fibre which 25 is hand held in a fibre chuck. The diode is operated in continuous mode at 75 mW during the laser soldering. Because this laser is Class 3b, and is not eye safe, protective glasses must be worn at all times when using this laser.

30 A suitable protein solder is a mixture of water, albumin and indocyanine green (ICG) dye (Becton Dickinson, Missouri). Indocyanine green has a maximum absorption coefficient at a wavelength of 805 nm of $2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. The percentages of albumin and dye compared 35 to the water were 110% and 0.6% respectively for fluid solder. 210% albumen was used in preparing solder

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strips. It is notable that ICG dye appears to preferentially bind with the albumin ensuring that heat is efficiently transferred to denature the protein solder.

5 Laser Soldering Technique

When conducting the surgery an operating microscope or some form of magnification is preferable. For a laser solder repair of a tubular join a section of thin gauze material is placed under the join to assist in a rotation 10 technique. The tissue edges are prepared in accordance with standard techniques for the tissue type and geometry of the repair.

Using micro forceps the edges are aligned and butted together. A 2 mm long stripe of fluid solder is 15 "painted" longitudinally across the junction of the edges using a 30 gauge needle freshly coated in the solder. Alternatively a strip solder is laid across the join using microforceps. The solid strip repair method is simpler. A solid strip is held in special microforceps 20 and placed across the junction parallel to the length of the structures to be joined. The laser output is then directed at the solid strip and the solid solder changes colour signalling denaturation which causes it to adhere to the underlying tissue membrane. The process is 25 repeated with further strips to ensure a strong union of surface.

The diode laser output from the 100 μm optical fibre is then used in a 30 second continuous pass to denature 30 solid solder into a strip weld. At a diode output power of 75 mW, the solid solder strip turns brown on the surface and opaque underneath from the single pass, signalling denaturation. When using fluid solder denaturation occurs more quickly. A two second laser pass can be sufficient to denature the fluid solder. 35 Generally a second layer of fluid solder is applied to the strip in order to increase the strength of the weld. and the two second laser pass is repeated. The gauze

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under the join is then used with the micro-forceps to rotate the join so that other strips can be applied.

Preparation of Fluid Protein Solder

Composition: - Albumin (fraction V powder from Sigma, St. Louis, Missouri) at least 100 % to 110% by weight compared to water.

5 - Indocyanine Green (Becton Dickinson, Missouri) approximately 0.6% by weight compared to water.

10 - Water (injection grade)

Procedure: A solution of ICG in water was prepared in a minitube. The albumin was added to the tube. The albumin and solution were mixed using a vortex mixer. This causes the protein structure to change leading to 15 linkage of protein molecules to each other rather than to water molecules.

Preparation of Solid Protein Solder

Composition: - Albumin (fraction V powder from Sigma, St. Louis, Missouri) 210% by weight 20 compared to water

- Indocyanine Green (Becton Dickinson, Missouri) approximately 0.6% by weight compared to water

- Water (injection grade)

25 Procedure: The ICG was dissolved in the water and the albumin was added to this solution in a minitube. This combination was mixed using a vortex mixer and a needle. The combination was mixed (for approximately 3 minutes) until it became a homogenous, malleable, green paste.

30 The phase of the mixture changed under this mixing technique to provide an almost solid composition with mainly protein to protein linkages rather than protein to water linkages. The system is no longer a solution at this stage. The protein paste was malleable and could be 35 cut into strips for up to about 30 minutes after mixing.

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After this time the paste hardened due to dehydration and became too hard to cut.

The resulting strips were between 50 and 100 μm in thickness, about 0.6 mm wide and 1.5 to 3.5 mm long. It 5 will be understood that where the strips are used in mending nerve fascicles that the desired width and length are dictated by fascicle dimensions. The width, thickness and length mentioned here are those suitable for use with a rat tibial nerve which has a diameter of 10 0.2 to 0.8 mm. The ratio of strip width to nerve circumference is typically:

Width \sim 1/5 circumference

EXAMPLE 1

A 100 μm core optical fibre-coupled 75 mW diode 15 laser operating at a wavelength of 800 nm has been used in conjunction with a protein solder to stripe weld severed rat tibial nerves, reducing the long operating time required for microsurgical nerve repair. Welding is produced by selective laser denaturation of the protein 20 based solder which contains the dye indocyanine green. Operating time for laser soldering was 10 \pm 5 min. (n=24) compared to 23 \pm 9 min. (n=13) for microsuturing. The laser solder technique resulted in patent welds with a tensile strength of 15 \pm 5 g, 25 while microsutured nerves had a tensile strength of 40 \pm 10 g. Histopathology of the laser soldered nerves, conducted immediately after surgery, displayed solder adhesion to the outer membrane with minimal damage to the inner axons of the nerves. An *in vivo* study, with a 30 total of fifty-seven adult male wistar rats, compared laser solder repaired tibial nerves to conventional microsuture repair. Twenty-four laser soldered nerves and thirteen sutured nerves were characterised at three months and showed successful regeneration with average 35 Compound Muscle Action Potentials (CMAP) of 2.4 \pm 0.7 mV and 2.7 \pm 0.8 mV respectively. Histopathology of the *in vivo* study, confirmed the comparable regeneration of

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axons in laser and suture operated nerves. A faster, less damaging and long lasting laser based anastomotic technique is presented.

Materials and Methods

5 1. Animals

A total of fifty-seven young adult male Wistar rats weighing between 400 and 550 g at the outset were used in this study. Thirty-four rats received laser solder repair and the remaining twenty-three 10 received standard microsuture repair as detailed below. Five rats of each repair method were used for tensile strength measurements and light microscopy immediately after surgery and the remaining thirty-seven rats were subjected to a 15 study of functional recovery using electrophysiology and histopathology.

2. Laser solder system

To denature the protein solder, a GaAs/GaAlAs laser diode with a nominal power of 250 mW (Spectra Diode Labs, San Jose, California) was used. The 20 laser light was coupled into a 100 μm diameter core optical fibre which was hand held in a fibre chuck. The diode laser was mounted on a heat sink, and the diode current and temperature were controlled by a 25 SDL-800 diode driver. The diode was operated in continuous mode at 75 mW during the laser soldering, corresponding to a maximum power density of 955 W/cm at the tissue. The laser output power was measured 30 with a Scientech (Boulder, Colorado) power meter. Because this laser is Class 3b, and is not eye safe, protective glasses were worn at all times when using this laser.

The solder used in this study was an albumin based protein mixture, also containing indocyanine 35 green (ICG) dye (Becton Dickinson, Missouri). Indocyanine green has a maximum absorption

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5 coefficient at a wavelength of 805 nm of $2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. It is notable that this dye appears to preferentially bind with the proteins ensuring that heat is efficiently transferred to denature the protein solder.

3. Surgery

10 Anaesthesia was maintained during surgery using a mixture containing Fluothane (4% during induction, 2% thereafter) in O₂ (1L/min). Using a OPMI 7 operating microscope (Zeiss, West Germany) the sciatic nerve of the left leg was exposed at the sciatic notch so that the nerve branches could be distinguished. The tibial branch, just below the 15 sciatic notch, was exposed from the surrounding subcutaneous tissue for a length of 1 cm. For a laser solder repair, a section of thin gauze material was placed under the tibial nerve to assist in rotation of the nerve, and for the suture repair, a section of plastic was placed under the nerve to allow easier suturing. The tibial nerve was then 20 severed with serrated micro-scissors and left for 3 minutes for the normal extrusion of axoplasm to occur. This was then trimmed with the serrated micro-scissors as required, after which the nerve 25 was repaired with either four laser solder strips or four 10-0 perineurial sutures.

30 The laser solder method involved aligning both stumps of the severed nerve with micro-forceps then a 2 mm long strip of solder was "painted" longitudinally across the junction of the severed ends using a 30 gauge needle freshly coated in the solder (Figure 3a). The diode laser output from the 35 100 μm optical fibre was then used in a continuous two second pass to denature the solder into a strip weld. At a diode output power of 75 mW, the solder was observed to turn brown on the surface and opaque underneath from the single pass, signalling

denaturation. A second layer of solder was applied to the strip and the two second laser pass was repeated. The gauze under the nerve was then used with the micro-forceps to rotate the nerve so that three other two layered stripes could be applied, each approximately 90° apart.

10 Seven rats were operated with a more advanced version of the organic solder, which is still an albumin based protein mixture but it has the advantage to be dehydratated and cut into solid rectangular strips (Figure 9). The average surface area of the solder strips was $1.5 +\text{-} 0.5 \text{ mm}^2$ and the thickness was $0.15 +\text{-} 0.01 \text{ mm}$. Four strips were positioned along the tibial anastomized nerve and then radiated with the same procedure adopted for the fluid solder. The solid strip was fused with the perineurium of the tibial nerve by the laser radiation, joining the extremities of the sectioned nerve.

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20 For all operation the time of anastomosis was recorded and a photographic record was taken for later reference. The animals were placed in their cages with no restriction of movement for 3 months.

4. Immediate Measurement of Tensile Strength and
25 Histopathology

In ten of the operated rats, the 1 cm long section of the laser and suture repaired nerves was harvested immediately for tensile strength measurements. Fine silk was tied to each end of the tibial nerve. One end was then attached to a calibrated force transducer (FT30C, Grass Instruments, Quincy, Mass) and the other to a screw driven translator (Figure 8) As the screw was turned the translator would stretch the nerve in a slow and steady manner. The applied tension was observed on an oscilloscope connected to the output of the force transducer. Tension was applied until

- 19 -

the nerve separated, and the breaking force was recorded. The nerves were kept moist, as upon drying, the tensile strength can be increased.

5 For light microscopy the anastomosis site of the tibial nerves were fixed in 5% formalin, alcohol dehydrated, imbedded in paraffin, longitudinally sectioned and stained with either Masson's trichrome or Giemsa.

10 5. Functional Assessment : Histopathology and Electrophysiology

15 Three months post operatively the rats were reanaesthetised using the method described in section 3. The site was exposed and the anastomosis of the tibial nerve observed. The two other branches of the sciatic nerve, the peroneal and sural nerves were then severed so that only the tibial nerve branch of the sciatic nerve could conduct electrical stimulation of the sciatic nerve to the muscles of the hind foot. Two days later the 20 rats were positioned on their side and insulated from the table by a folded surgical drape. An infrared lamp was used to maintain their rectal temperature above 36°C.

25 A clinical electromyograph (Cadwell Sierra EMG/EP) was used for stimulation and recording. Two 25 gauge stimulating electrodes were placed 10mm apart on each side of the sciatic nerve above the sciatic notch, near the hip. The nerve was 30 activated using rectangular pulses (0.1 to 0.3 ms; 0 to 30 mA; 1 Hz). Compound muscle action potentials (CMAPs) were recorded from the plantar muscles of the foot in response to supramaximal stimulation of the sciatic nerve. A set of three recording electrodes were used. A 25 gauge ground electrode was inserted subcutaneously between the stimulating and recording electrodes 1,2. A 30 gauge reference electrode was inserted into the heel pad and a 30

- 20 -

gauge recording electrode was inserted into the plantar muscles of the foot. The CMAPs were recorded and processed to determine their negative wave peak value.

5 Histopathology of the sutured and laser soldered nerves, was conducted after the Electrophysiology test with the same procedure as adopted in section 4.

Results

10 At the completion of surgery all anastomoses were successful. The operating procedure was found to be easier for laser soldering than for microsuturing. This resulted in the shorter operating times for laser solder repairs {10 ± 5 min (n=24)} than {23 ± 9 min (n=13)} for 15 microsuture repairs. The tensile strength of five laser solder repaired nerves immediately after the operation was 15 ± 5 g and the tensile strength of the microsutured nerves, 40 ± 10 g.

20 Histopathological examination of the anastomosis sites immediately after surgery demonstrated that the albumin and ICG dye based laser solder does bond well with the outer membrane of the nerve, the perineurium, while the inner axons remain unheated. In Figure 10a, a tibial nerve fascicle weld produced by the diode laser 25 and albumin/ICG dye solder is shown in section. Both the protein solder and the perineurium have denatured forming the bond. On the lower side of the bond, the axoplasm has its normal wavy structure. Note that since heating is concentrated at the dye, only denaturation of the 30 solder and adjacent perineurium occurs.

One of the promising aspects of laser anastomosis is the potential for reduced damage to the axoplasm by removing the need for sutures. A section showing the 35 effect of microsuturing nerve fascicles using 10/0-nylon is shown in Figure 5b. This section stained with Giemsa, displays axon extrusion at the join, as well as localised perineurial and axonal damage due to the suture.

Histopathology at 3 months shows regeneration of myelinated axons in laser nerve repairs (Figure 10a), with no discontinuity of either the fibers and their sheaths, or the fibrous perineurium. No evidence is seen 5 of inflammation or myelin phagocytosis. Full restoration, as assessed by light microscopy, of the histologic integrity of the tibial nerve has been achieved by the laser weld.

The sutured nerves also show successfull anastomosis 10 with myelinated axon regeneration, however, it is still evident that the nylon thread is surrounded by fibrous tissue, which creates an obstacle to the directionality of the regenerated axons (Figure 10b).

The electrophysiological measurements of the *in vivo* 15 study were performed on twenty-four laser solder repaired rats and thirteen microsuture repaired rats having three months recovery. Of this group all twenty-four laser solder anastomoses were patent as were the thirteen microsuture anastomoses. The average amplitude of the 20 muscle action potentials, resulting from supramaximal stimulation of the nerve above the repair site was 2.4 +/- 0.7 mV for the twenty-four laser soldered tibial nerves and 2.7 +/- 0.8 mV for the thirteen microsutured nerves. The normal muscle action potential produced by 25 stimulating the tibial nerve supramaximally was recorded at 8.7 +/- 3 mV from ten rats (Figure 11).

Discussion

Clinically, when a major peripheral nerve is severed, forty or more fascicles may need to be 30 individually rejoined. With three or four microsutures per fascicle, suturing tends to be prolonged, as it must be meticulous. In a nerve graft, where two anastomoses are needed, the suturing time is doubled. We have sought a suitable method of nerve anastomosis that could at 35 least duplicate the end result but was significantly faster than the present hand sewing microsuture technique. A bonus of the described laser soldering

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method was the demonstrated lack of change to the axonal components beneath the denatured perineurial layer seen immediately after surgery. Three months later comparable regeneration was demonstrated by electrophysiological 5 nerve conduction studies.

INDUSTRIAL APPLICABILITY

The present invention has application in the field of surgery where it is of application in joining together tissue edges, in end to end, side to end and side to side 10 applications.

REFERENCES

- 1) R. Malik, S. Ho and D.B. Church: A new method for recording and analysing evoked motor potential from dogs. *Journal of Small Animal Practice* (1989) 30, 13-19.
- 5
- 2) R. Malik, S. Ho: Motor nerve conduction parameters in the cat. *Journal of Small Animal Practice* (1989) 30, 396-400.
- 10 3) Laser activated protein bands for peripheral nerve repair. A Lauto, R Trickett, R Malik, J Dawes, E Owen. European Biomedical Optics Week - BIOS Europe 195 12-16 September 1995 (Proceeding in Press)

THE CLAIMS

1. A method for joining tissue comprising:
aligning and abutting edges of the tissue to be joined;
5 applying a biodegradable, biological solder or an analogue thereof, as herein defined, across the edges; and
exposing the solder to an energy source under conditions which provide transfer of energy from the
10 source to the solder to cause the solder to bond to the tissue surface adjacent the edges to provide a weld holding the edges together.
2. The method of claim 1 wherein the tissue is nerve tissue and the edges are ends of a peripheral nerve
15 fascicle or a nerve fascicle and nerve graft material, and welding is not effected along the line of discontinuity so as to protect nerve tissue from damage.
3. The method of claim 1 wherein the tissue is an anastomosis of a biological tube including veins,
20 arteries, lymphatic, vasa efferentia, fallopian tubes, bile ducts, tubes of the alimentary canal, the ureter, the urethra, tear ducts or bronchi, and wherein a hollow cylinder of the solder is inserted into the tube between the discontinuous ends prior to the application of solder
25 to the external surfaces of the tube being joined.
4. A method according to claim 3 wherein the discontinuous ends are held in place while energy is applied to the cylinder within the tube to cause the cylinder to bond with the inner surface of the tube.

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5. The method of claim 1 wherein the tissue is a repair of an incision or tear of a biological organ including kidneys, liver or spleen, or of a biological surface such as the peritoneum or skin.

5 6. A method for repairing a discontinuity in a tissue surface comprising:

applying a biodegradable, biological solder or an analogue thereof as herein defined to the discontinuity; and

10 exposing the solder to an energy source under conditions which provide transfer of energy from the source to the solder to cause the solder to bond within itself and to the tissue surface to provide a weld holding the solder and tissues surrounding the

15 discontinuity together.

7. The method of claim 1 or 2 wherein a first strip of the solder is applied and exposed to the energy source, then a second strip is applied close to the first strip and exposed to the energy source and this process 20 is repeated to provide a plurality of strip welds.

8. The method of any one of claims 1 to 7 wherein the biodegradable, biological solder is a protein solder.

9. The method of claim 8 wherein the protein solder 25 is a solid or a fluid solder.

10. The method according to any one of claims 1 to 9 wherein the energy source is a laser.

11. A method according to any one of claims 1 to 10 wherein the solder incorporates a substance which 30 absorbs the energy from the energy source highly compared to the tissue.

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12. A method according to claim 11 wherein the substance is a dye.

13. A fluid protein solder composition comprising 100 to 120 mass % protein relative to water, 5 and a suitable solvent for the protein.

14. A fluid protein solder composition according to claim 13 comprising 100 to 110 mass % protein relative to water, and a suitable solvent for the protein.

10 15. A substantially solid protein solder comprising 120 to 230 mass % protein relative to water, and a suitable solvent for the protein.

15 16. A substantially solid protein solder comprising 170 to 230 mass % protein relative to water, and a suitable solvent for the protein.

17. A substantially solid protein solder comprising 210 mass % protein relative to water, and a suitable solvent for the protein.

18. A kit for joining tissues comprising, in a 20 preferably sterile pack, a plurality of strips and/or shapes of a protein solder according to any one of claims 15 to 17.

19. A fluid protein solder composition comprising 100 to 120 mass % protein relative to water, 25 and a suitable solvent for the protein, when used in a method according to any one of claims 1 to 10.

20. A fluid protein solder composition according to claim 19 further comprising a substance 30 which absorbs the energy from the energy source highly compared to the tissue.

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21. A fluid protein solder composition according to claim 20 wherein the substance is a dye.

22. A substantially solid protein solder comprising 120 to 210 mass % protein relative to water, 5 and a suitable solvent for the protein, when used in a method according to any one of claims 1 to 10.

23. A substantially solid protein solder according to claim 22 further comprising a substance which absorbs the energy from the energy source highly 10 compared to the tissue.

24. A substantially solid protein solder according to claim 23 wherein the substance is a dye.

AMENDED CLAIMS

[received by the International Bureau on 10 May 1996 (10.05.96); original claims 1, 2, 13-19 and 22 amended; new claims 25-29 added; remaining claims unchanged (5 pages)]

1. A method for joining tissue comprising:
 - aligning and abutting edges of the tissue to be joined;
 - 5 applying a biodegradable, biological solder or an analogue thereof, as herein defined, across the edges as one or more transverse strips; and
 - 10 exposing the solder to an energy source under conditions which provide transfer of energy from the source to the solder to cause the solder to bond to the tissue surface adjacent the edges to provide a weld holding the edges together, wherein when more than one strip is applied, the strips are spaced apart to permit natural co-appration of the join.
- 15 2. The method of claim 1 wherein the tissue is nerve tissue and the edges are ends of a peripheral nerve fascicle or a nerve fascicle and nerve graft material, and welding is not effected along the line of discontinuity so as to protect the nerve tissue from damage.
- 20 3. The method of claim 1 wherein the tissue is an anastomosis of a biological tube including veins, arteries, lymphatic, vasa efferentia, fallopian tubes, bile ducts, tubes of the alimentary canal, the ureter, the urethra, tear ducts or bronchi, and wherein a hollow cylinder of the solder is inserted into the tube between the discontinuous ends prior to the application of solder to the external surfaces of the tube being joined.
- 25 4. A method according to claim 3 wherein the discontinuous ends are held in place while energy is applied to the cylinder within the tube to cause the cylinder to bond with the inner surface of the tube.

5. The method of claim 1 wherein the tissue is a repair of an incision or tear of a biological organ including kidneys, liver or spleen, or of a biological surface such as the peritoneum or skin.

5 6. A method for repairing a discontinuity in a tissue surface comprising:

applying a biodegradable, biological solder or an analogue thereof as herein defined to the discontinuity; and

10 exposing the solder to an energy source under conditions which provide transfer of energy from the source to the solder to cause the solder to bond within itself and to the tissue surface to provide a weld holding the solder and tissues surrounding the 15 discontinuity together.

7. The method of claim 1 or 2 wherein a first strip of the solder is applied and exposed to the energy source, then a second strip is applied close to the first strip and exposed to the energy source and this process 20 is repeated to provide a plurality of strip welds.

8. The method of any one of claims 1 to 7 wherein the biodegradable, biological solder is a protein solder.

9. The method of claim 8 wherein the protein solder 25 is a solid or a fluid solder.

10. The method according to any one of claims 1 to 9 wherein the energy source is a laser.

11. A method according to any one of claims 1 to 10 wherein the solder incorporates a substance which 30 absorbs the energy from the energy source highly compared to the tissue.

12. A method according to claim 11 wherein the substance is a dye.

13. A fluid protein solder composition comprising 100 to 120 mass % protein relative to water as 5 a starting amount prior to mixing, and a suitable solvent for the protein.

14. A fluid protein solder composition according to claim 13 comprising 100 to 110 mass % protein relative to water as a starting amount prior to 10 mixing, and a suitable solvent for the protein.

15. A substantially solid protein solder comprising 120 to 230 mass % protein relative to water as a starting amount prior to mixing, and a suitable solvent for the protein.

15 16. A substantially solid protein solder comprising 170 to 230 mass % protein relative to water as a starting amount prior to mixing, and a suitable solvent for the protein.

17. A substantially solid protein solder 20 comprising 210 mass % protein relative to water as a starting amount prior to mixing, and a suitable solvent for the protein.

18. A kit for joining tissues comprising, in a preferably sterile pack, a plurality of strips and/or 25 shapes of a protein solder according to any one of claims 15 to 17.

19. A fluid protein solder composition comprising 100 to 120 mass % protein relative to water as a starting amount prior to mixing, and a suitable solvent 30 for the protein, when used in a method according to any one of claims 1 to 10.

20. A fluid protein solder composition according to claim 19 further comprising a substance which absorbs the energy from the energy source highly compared to the tissue.

5 21. A fluid protein solder composition according to claim 20 wherein the substance is a dye.

10 22. A substantially solid protein solder comprising 120 to 210 mass % protein relative to water as a starting amount prior to mixing, and a suitable solvent for the protein, when used in a method according to any one of claims 1 to 10.

15 23. A substantially solid protein solder according to claim 22 further comprising a substance which absorbs the energy from the energy source highly compared to the tissue.

24. A substantially solid protein solder according to claim 23 wherein the substance is a dye.

25. A substantially solid protein solder according to claim 15 wherein the protein is albumin.

20 26. A fluid protein solder composition according to claim 13, wherein the protein is albumin.

27. A substantially solid protein solder according to claim 15 wherein the protein is one having a high proportion of β sheet structure.

25 28. A substantially solid protein solder according to claim 27 wherein the protein has less than about 10% by weight α helical content.

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29. A substantially solid protein solder according to claim 15 with the proviso that the composition is not one consisting of 70.3% by weight collagen, 16.9% by weight plasticizer and 9% by weight
5 water.

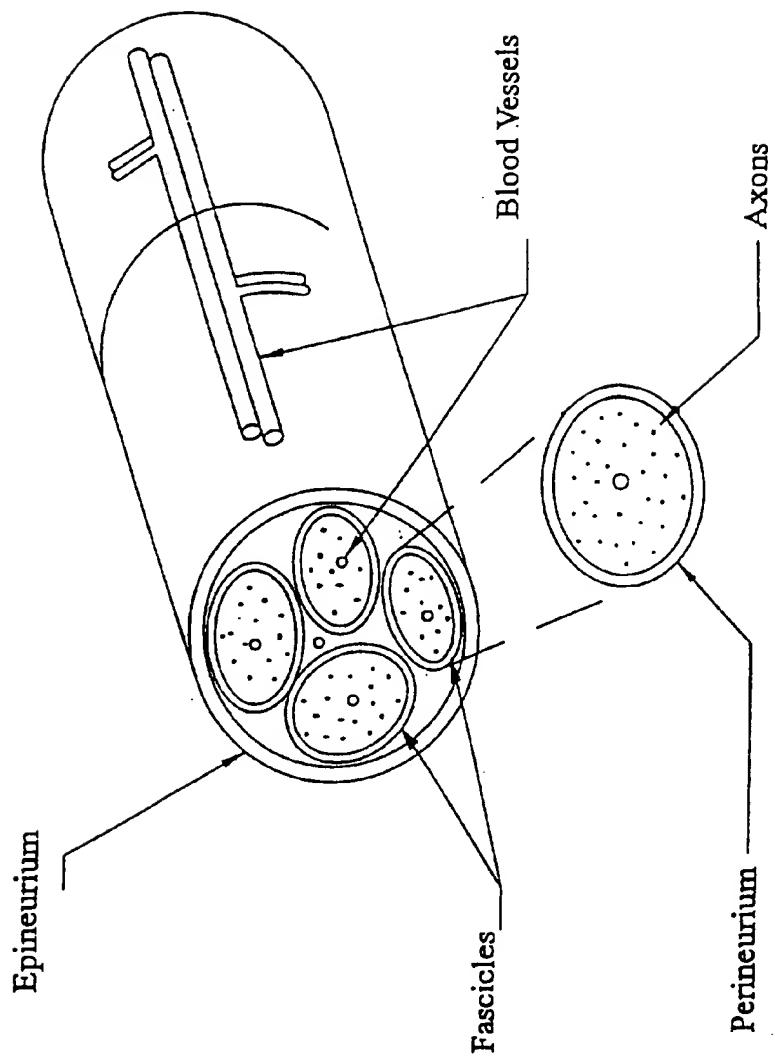


Figure 1

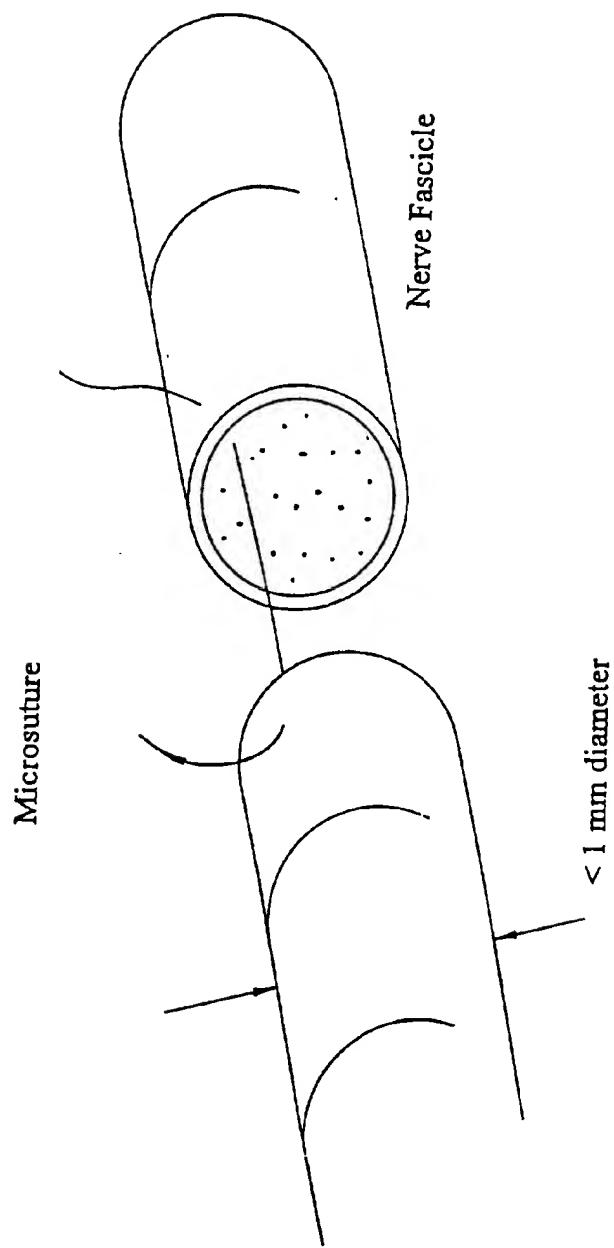


Figure 2

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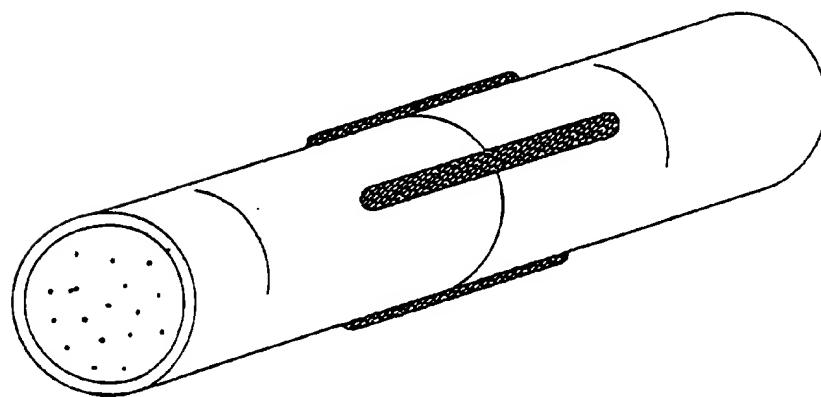
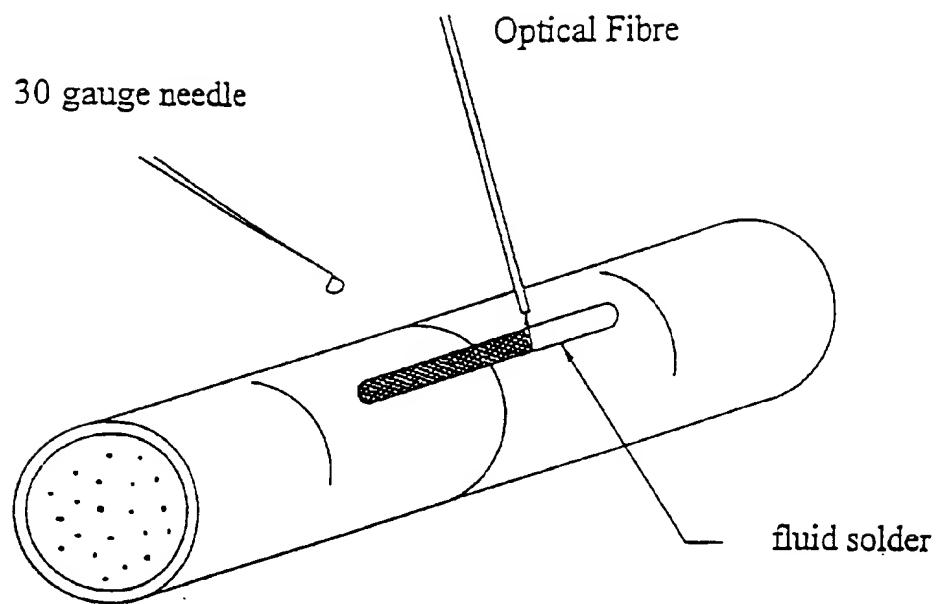


Figure 3a

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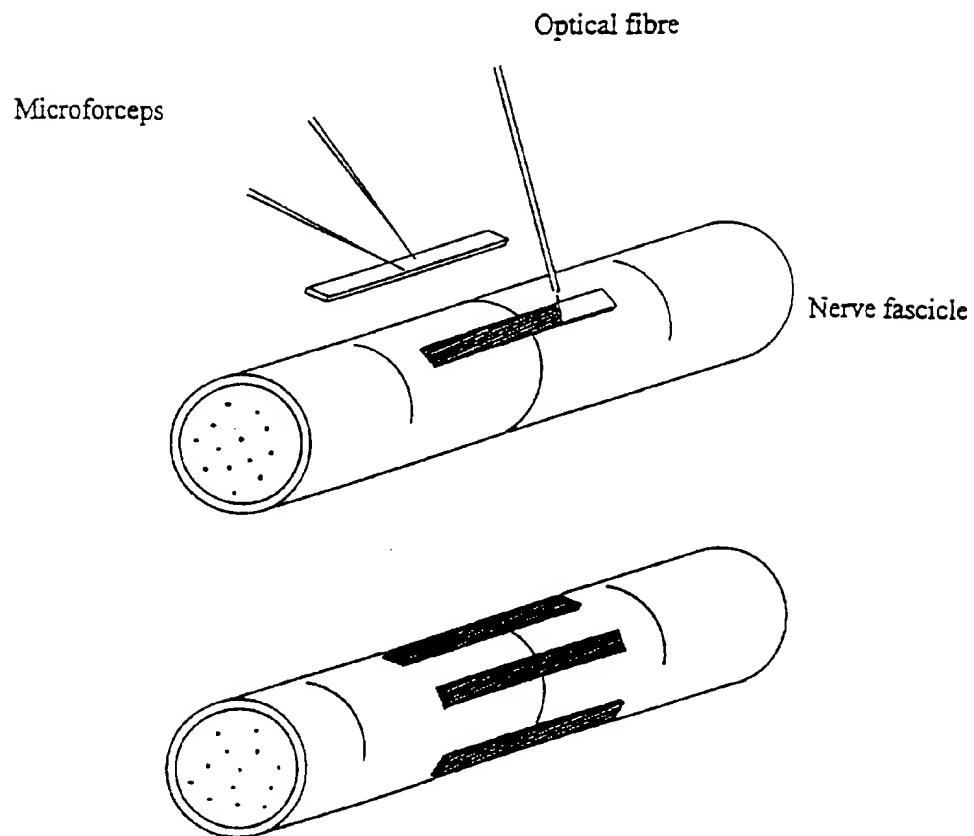


Figure 3b



Figure 4b



Figure 4a



Figure 5b



Figure 5a

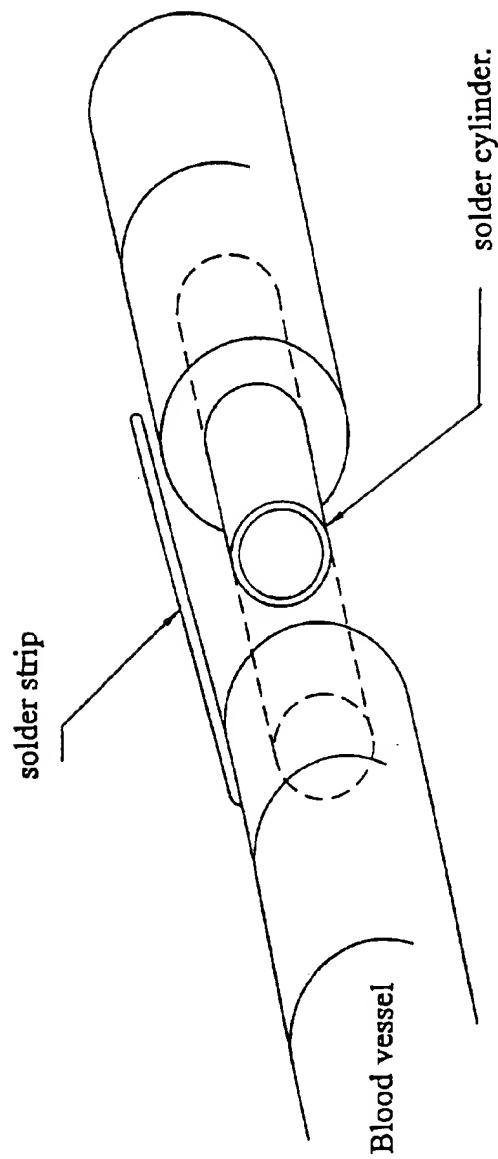


Figure 6

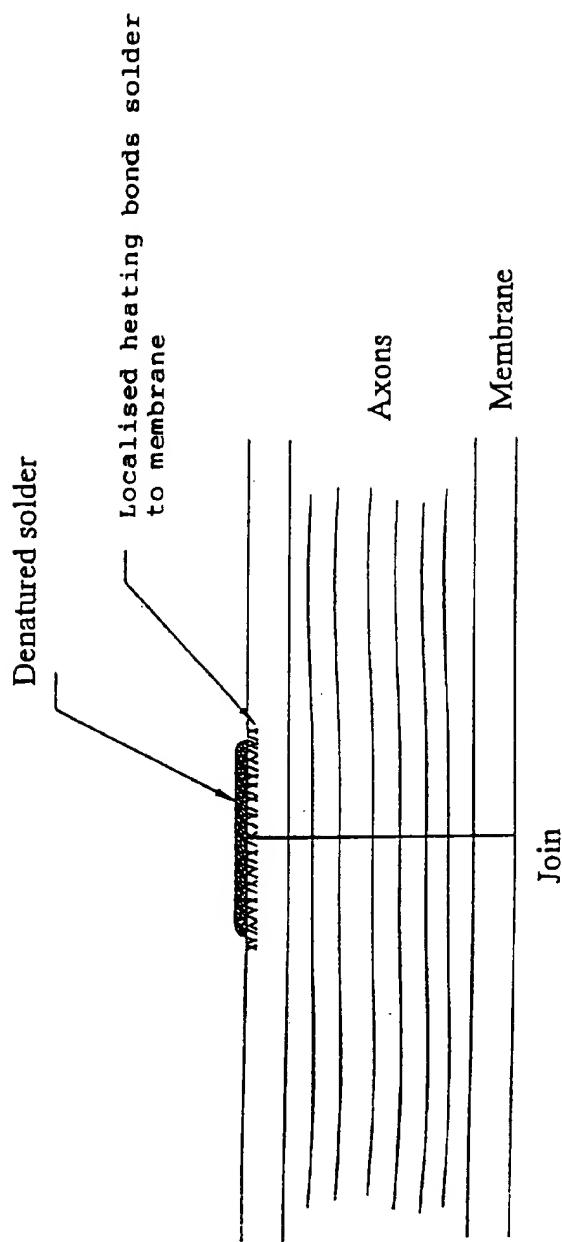


Figure 7

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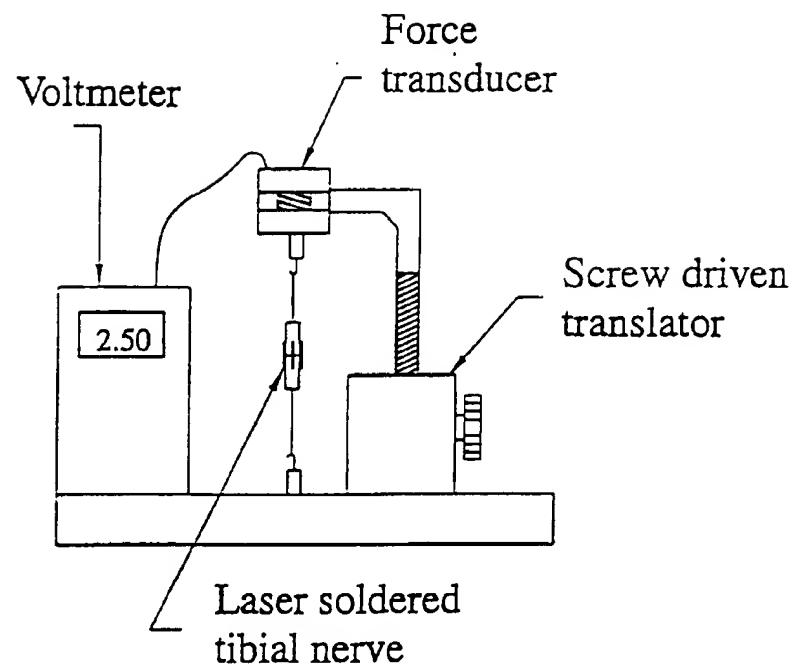


Figure 8

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Figure 9

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Figure 10b



Figure 10a

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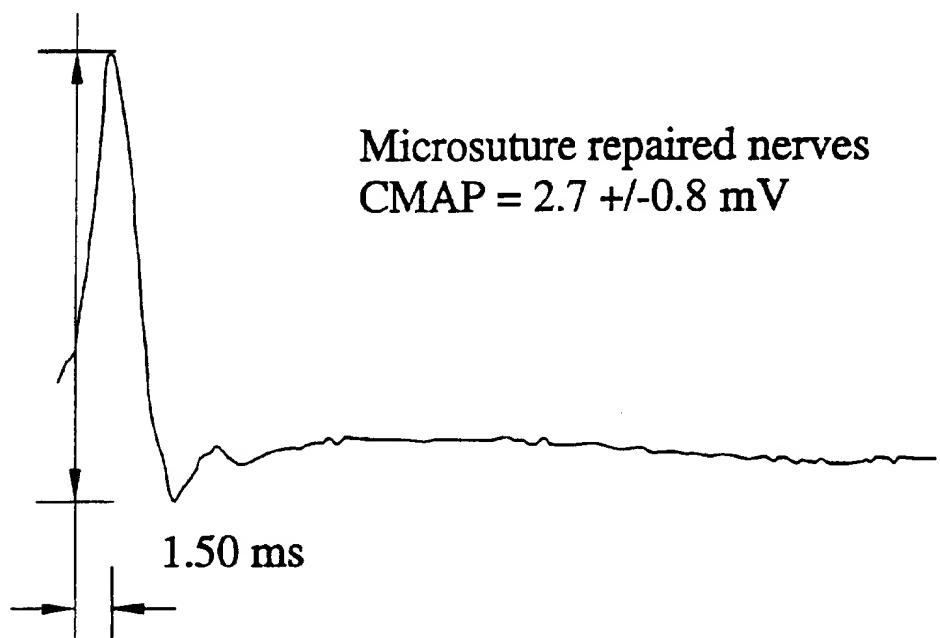
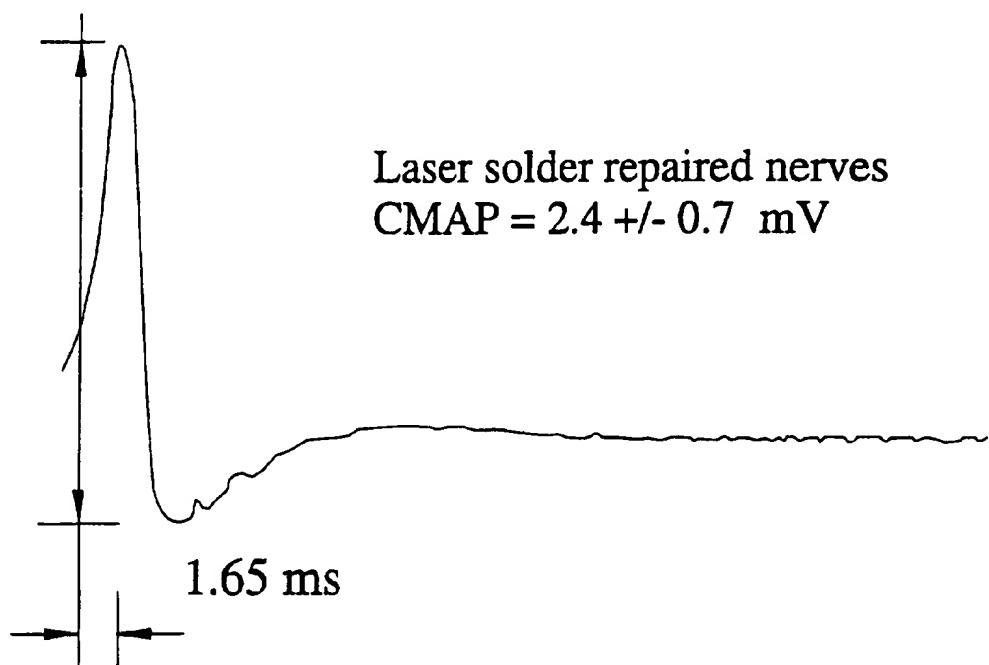


Figure 11

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00024

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl⁶: A61B 17/03

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC A61B 17/03, 17/36, 19/00 A61N 5/06Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above, A61L 25/00Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT} tissue and (weld or solder)
JAPIO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92/14513 A (INTERFACE BIOMEDICAL LABORATORIES CORP) 3 September 1992 page 3 line 22- page 4 line 10, Figure 3 page 8 line 33- page 9 line 2 page 20 lines 5-13	1, 3-12
A	page 9 lines 29-33	13-18
X	WO 91/04073 A (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 4 April 1991 page 3 lines 1-26	1, 5, 6, 8-12

 Further documents are listed in the continuation of Box C See patent family annex

• Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search
7 March 1994

Date of mailing of the international search report

14-03-96

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00024

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Page 3 line 27- page 4 line 5	13-18
P,X	US 5409481 A (POPPAS et al) 25 April 1995 column 3 lines 57-66	1, 5, 6, 8-12
A	US 5292253 A (LEVY) 8 March 1994 column 1 lines 46-55	1, 6
A	US 5330974 A (PINES et al) 19 July 1994 whole document	13-18

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00024

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	9214513	AU	14442/92	CA	2103727	EP	572526
		US	5156613				
WO	9104073	AU	64071/90	EP	491794	IL	95664
US	5409481	US	5334191				
US	5292253						
US	5330974	AU	62343/94	WO	9420524		

END OF ANNEX